brought to you by CORE

QÜESTIIÓ, vol. 22, 2, p. 365-378, 1998

ANALYSIS OF SURVEY DATA INVESTIGATING THE MALARIAL ENDEMICITY OF A MIXED TRIBAL POPULATION OF BIHAR, INDIA

T.K. BASU S. GANGULY S.K. SARKAR Indian Statistical Institute*

R. ARNAB

University of Durban**

A section of the mixed tribal population of the Singhbhum district, Bihar, India is declared malaria epidemic zone. The tribal population of several generation is known to be suffering from malaria. A survey based on crosssectional data analysis, was conducted on the mixed tribal population for one month. The purpose of this study was to investigate the health status using collected blood samples. The main focus is on the comparative roles of the «defense mechanism» and «vitality factor» of the human system in context to the malarial infection. By gradual elimination of the blood parameters by statistical analyses, the «vitality» parameters probing malarial endemicity are assessed with a view to predicting the epidemic.

The main findings from this survey are (i) of the selected twenty two parameters of blood, albumin, total cholesterol, total protein, β -Globulin, γ -Globulin, Immuno globulin G seem to have some predictive capacity with respect to the malarial endemicity of the tribal people and (ii) Categorical variables like blood groups and sex are comparatively less important for prediction of malaria.

Keywords: Vitality factors; defense mechanism; two-way Anova; discriminant function.

^{*} Biometry Research Unit. Indian Statistical Institute. 203 Barrackpore Trunk Road. Calcutta 700 035, India.

^{**} Department of Statistics. University of Durban, South Africa.

⁻ Received January 1997.

⁻ Accepted November de 1997.

1. INTRODUCTION

The Indian Council of Medical Research, a leading medical research concern of India, identified a few villages of tribal habitat of the Singhbhum district of Bihar (India) as a malarial epidemic zone. The people of these villages show evidence of malarial suffering for several generations. A study was undertaken to investigate the malarial endemicity by checking the blood samples obtained from a mixed tribal population of the Singhbhum district. The investigation was executed by the Indian Council of Medical Research in conjunction with the Biometric Unit, Indian Statistical Institute.

Malaria depends upon two principal factors- (a) vitality and (b) defense mechanism, regulated by the circulation of blood. Vitality is controlled by total, free and ester cholesterol, albumin, red blood corpuscles (RBC) and haemoblobin, and the defense mechanism is controlled by immunoglobulins (IgG, IgA and IgM), white blood corpuscles (WBC), monocytes, nutrophils, eosinophils and lymphocytes. Lymphocytes are subdivided into T and B lymphocytes. In addition, the role of globulin-containing fractions is also prominent on this aspect. Under malarial conditions, RBC, haemoglobin and albumin decrease whereas WBC, neutrophils and globulins tend to increase. This is a very broad overview of this complex mechanism. In this context, a brief description of the biology of malaria is presented.

1.1. Biology of Malaria

Malaria is caused by a parasite protozoan infection. Four different species of the genus Plasmodium are known to infect humans. In the tropics, Plasmodium falciparum is most prevalent. The life cycle of the parasite is an interaction between a female mosquito of the genus Anopheles (the vector) and the human host. Transmission of disease occurs by a bite of the infectious mosquito. The parasite migrates to the liver, remains in the latent stage for several days while replicating. Ultimately, there occurs a penetration of host RBC with an asexual replication within the parasites which results in the lysis of the cells. Malarial symptoms are occurred by this asexual parasites in the blood. When there is a fresh case of mosquito biting, a sexual stage, called gametocytes develops (from the asexual parasites) which is responsible for transmission of parasites from the host. These transmitted parasites fertilize in mosquito gut, replicate and a new cycle of transmission starts. These types of epidemiological surveys are generally based on blood smears in which one can observe both the asexual parasites and gametocytes. Classifications of blood smears are generally done in terms of the appearance/absence of parasite or in terms of its density. According to above, there are actually three classes of population related to the malarial status- susceptibles, the proportion that are uninfected (Class 1); infecteds, the proportion with infection (Class 2); and immunes, the proportion with asymptotic infection which is not prominent (Class 4). The Febrile Class i. e. Class 3 represented subjects with unidentified fever, not malaria (as confirmed by slide test).

1.2. Main Findings

Subsequent analysis of data gives some indication that the levels of albumin, total cholesterol, total protein, β - Globulin, γ -Globulin and IgG in the circulating system may be used for the prediction of malarial endemicity of the tribal population. The categorical variables like blood groups and sex are less important for the prediction of the malarial incidence.

2. SAMPLING DESIGN AND COLLECTION OF DATA

A simple random sample of 88 persons were selected from the list of volunteers from the tribal population. Among them, 45 were male and 40 were female. The selected individuals were invited at the Clinics of Kokda and Khanderber, organized by the Gram Vikas Kendra, a rural development centre of Tata Engineering and Locomotive Works, Jamshedpur, Bihar. The list of volunteers was prepared earlier by the Centre. 10 persons were invited daily in the Clinics serially from the chart. The average daily attendance was six. This is because most of the females did not respond owing to low literacy level, social-taboos or customs. The nature of nonresponse is quite random in nature and so this will not cause any bias in the experiment. However, the efficiency of the estimators will reduce because of the reduction of sample size. Relevant history regarding family members, total income of the family and other socio- economic data of the volunteers were collected by the Centre and were sent to the Clinics for record. They were mostly nonvegetarians residing in huts as usually observed in tribal villages. According to their family income, they belonged to the «below poverty-level» group.

2.1. Experimental protocol

Subjects, as instructed earlier, came in post-absorptive stage (overnight fasting) in the Clinics between 9 to 10 am. The attending physician checked the weight, pulse and blood pressure of each individual and drew blood from their bracial vein for subsequent testing. 10ml of blood was taken in an aseptic condition for the biochemical, haematological and immunological tests. Out of this 10ml blood, 4ml was kept in a sterilized glass vial with the sequestering agent. Blood smear was prepared on two glass slides, one thick (for the detection of Malarial parasite) and the other thin

(for differential count of the blood cells). The remaining portion of blood was taken in a sterilized test tube and was allowed to clot for release of serum.

The electrophoretic separation of different protein fractions was carried out in the line of Smithies(1955) with 1% agar and 0.1M Veronal Buffer (pH 8.6). The fractions of Protein- Albumin and Globulins were scanned by using a Densitometer. The relative percentage of different Protein fractions was measured by using a Planimetre.

The Immunodiffusion study was performed according to the standard method (Mancini et al, 1965). The diametre of the precipitating ring was measured in mm by using the 'Immunomeasure' scale. The standard curve was plotted on a double log graph paper using standard antigens supplied by M/s Immunodiagnostics Pvt. Ltd., Delhi. The Immunoglobulin fractions were calculated from the standard curve. The conventional methods for the estimation of Total protein (Lowry et al, 1951), Total, Free and Ester cholesterol(Wootton, 1974), Haemoglobin, RBC, WBC, Blood groups and Differential counts i.e.estimation of Leucocytes, Monocytes, Eosinophiles, Basophils (Davie and Lewis, 1984), T- and B- Lymphocytes (Blood, 1977) were followed (Summary tables 1A-1C). On the basis of the above pathological tests the population was post stratified into four Classes according to their malarial status.

Class 1. Normal: Those who had no fever currently and no past history of malaria within the last couple of years. Total number 20 (17 male and 3 female)

Class 2. Malarious: Those who turned up with symptoms of malaria and showed the malarial parasite positive in the 'slide test'. Total number 15 (11 male and 4 female)

Class 3. Febrile: Those who emerged with fever but no malarial parasite was identified in the 'slide test'. Total number 25 (15 male and 4 female).

Class 4. Dormant: Those who had recurring malaria during the last two years but showed no current malarial symptoms. Total number 28 (21 male and 7 female).

3. STATISTICAL ANALYSIS

A: Analyses of cardiovascular and blood parameters

The means of the following twenty one blood parameters (viz. Total Protein (TP), Total Chlesterol (Tch), Free Chlesterol (Fc), Ester Cholesterol (Ec), Red Blood Corpuscles (RBC), White Blood Corpuscles (WBC), Neutrophils (N), Lymphocytes (L), Eosinophils (E), Monocytes (M), Haemoglobin (Hb), Immuno- globulin G (IgG), Immunoglobulin A (IgA), Immunoglobulin M (IgM), Albumin (Alb), α_1 -Globulin (α_1), α_2 -Globulin (α_2), β -Globulin, γ -Globulin, T-cell (Tc), B-cell (Bc) and two cardio-

vascular parameters, namely, Pulse (P) and Blood Pressure, Systolic/Diastolic [Bp (Sys/Dia)] for the four classes of people (Normal, Malarious, Febrin and Dormant) were estimated through the sample means which are given in the tables 1A, 1B and 1C. The estimated standard errors (SR) of the estimates are also presented in these tables. The following table gives an idea of the general health condition of the tribal community under consideration.

Ta	bl	e	1

Parameters	Normal Range Indian Standard	mean	standard deviation		Proportion below normal range	Proportion above normal range	Proportion outside normal range	sample range min-max
P(c/min)	65-85	94.898	15.834	1.688	0	70.11	70.11	68-124
BP/sys	100-140	110.932	13.118	1.398	17.04	0	17.04	78-150
BP/dia	70-90	76.33	10.783	1.149	20.45	4.54	24.94	54-100
WBC(m/cmm)	4000-10000	7185.8	931.853	99.336	0	0	0	5000-9500
α ₁ (% Tp)	52-68	3.555	1.351	0.144	26.13	23.86	50	0.9-8.7
$\alpha_2(\% Tp)$	6.1-10.1	3.838	1.697	0.181	89.7	0	89.7	1-9.9
β(% Tp)	8.5-14.5	6.619	2.495	0.266	84.1	0	84.1	2.4-14.5
γ(% Tp)	10-21	18.991	5.38	0.537	4.54	27.27	31.88	6-42.9
IgG(mg%)	700-1500	2453.69	598.515	63.802	0	85.22	85.22	1205-3477
IgA(mg%)	90-450	193.818	18.698	1.993	0	0	0	152-237
IgM(mg%)	40-250	128.352	28.642	3.053	0	0	0	69-192
Alb(% Tp)	52-68	67.436	7.046	0.751	2.27	51.13	53.46	45.7-85.1
N(%)	60-65	56.125	5.858	0.624	65.9	6.81	72.72	42-68
L(%)	20-40	33.318	4.797	0.511	0	7.95	7.95	24-48
E(%)	1-3	8.148	4.268	0.455	0	87.45	87.45	1-19
M(%)	2-8	2.614	1.309	0.14	13.63	0	13.63	1-6
Hb(gm%)	14-16	11.063	1.231	0.131	100	0	100	8-12.8
Tc(%)	70-75	63.5	6.447	0.687	80.68	0	80.68	42-75
Bc(%)	15-20	24.08	5.126	0.546	1.13	70.45	71.59	12-38
Tp(gm%)	6-8	7.142	0.387	0.041	0	0	0	6.5-8
Tch(mg%)	150-280	126.773	22.951	2.447	82.95	0	82.95	80-184
Fc(mg%)	50-70	42.045	13.597	1.449	78.4	2.27	80.68	20-94
Ec(mg%)	95-210	85.886	22.21	2.368	69.31	0	69.31	26-140
RBC(m/cmm)	4000-10000	3.794	0.474	0.051	98.86	0	98.86	2.31-4.78

From the above table, we briefly comment on the average health condition as follows:

BP: Due to the simplicity of living and diet, the community under study maintained acceptably good BP levels.

RBC, Hb: Lower values indicate that most of the people are anaemic.

Tp, Tch, Fc, Ec: Lower values than the normal range is indication of low fat diets coupled with normal protein levels.

E: High concentration of eosinophils is generally correlated with parasitic infection in the people.

WBC, N, L, M: The levels of these parameters are acceptable in terms of facilitating immnuno-defence mechanism.

Summary Table: 1A

Class	Sample	Statistic	Р	BP/sys	BP/dia	TP	Tch	Fc	Ec	RBC	WBC
	size		(count/min)	(mm Hg)	(mm Hg)	(gm%)	(mg%)	(mg%)	(mg%)	(m/cumm)	(no/count)
		mean	91.6	112.6	77.7	7.08	136.2	39.2	102.5	4.062	7707.5
1	20	sd	16.28005	8.9241	8.97273	0.32031	21.7154	11.26765	14.42047	0.28971	816.13035
		se	3.64033	1.9955	2.00636	0.07162	4.85572	2.51952	3.22451	0.06478	182.19229
		mean	94.06667	108.67	7606	6.94666	96	38 63333	57.46667	3 72133	6736.66667
2	15	sd	15.48533	13.656	12.04326					0.48780	772.97405
		se	3.99829	3.526	3.10955	0.06172	2.58543	3.74506	3.59983	0.12595	199.58104
		mean	100.4	106.36	72.92	7.156	136.16	45.72	90.12	3.6264	7084
3	25	sd	15.09967	14.982	10.9724	0.43458	20.9908	13.92413	19.17982	0.43472	953.90984
		se	3.01993	2.9965	2.19448	0.08691	4.19816	2.74482	3.83596	0.08694	190.78197
		mean	92.78571	115.04	78.25	7 27057	128 142	42.67857	85 45420	2 70071	7144.64286
4	28		15.065	113.04	10.35659			42.07837	83.43429 17.97713		898.54438
4	28	sd	2.84701	2.263	10.35659		2.80475		3.39735	0.52002	898.54438 169.80892
		se	2.04/01	2.203	1.93721	0.07440	2.00475	2.33347	5.59/55	0.09827	109.60892

Summary Table: 1B

e me o sd se me o sd se	4.2 0.9 an 53 5.9	.45 3 225 3 045 0 .667 3 007 4	32.85 3.454 0.772 36.4 4.514	0.855	0.18 4.267 1.289		(%) 65 4.278 0.957 63.733 5.234 1.351	(%) 23.1 4.3 0.962 23.6 5.414 1.398
) sd se me 5 sd	4.2 0.9 an 53 5.9	225 3 045 0 .667 3 007 4	3.454 0.772 36.4 4.514	3.825 0.855 5.667 3.32	0.805 0.18 4.267 1.289	0.621 0.139 10.647 1.447	4.278 0.957 63.733 5.234	4.30.96223.65.414
se me 5 sd	0.9 an 53 5.9	045 0 .667 3 007 4	0.772 36.4 4.514	0.855 5.667 3.32	0.18 4.267 1.289	0.139 10.647 1.447	0.957 63.733 5.234	0.962 23.6 5.414
me sd	an 53 5.9	.667 3 907 4	36.4 4.514	5.667 3.32	4.267 1.289	10.647 1.447	63.733 5.234	23.6 5.414
sd	5.9	07 4	4.514	3.32	1.289	1.447	5.234	5.414
sd	5.9	07 4	4.514	3.32	1.289	1.447	5.234	5.414
se	1.5	525 1	1.165	0.857	0 333	0.374	1 351	1.398
					0.555	0.574	1.551	1.570
me	an 58	8 3	31.56	7.64	2.64	10.704	62.16	25.32
i sd	6.2	203 5	5.375	4.906	1.353	1.144	7.22	5.732
se	1.2	241 1	1.075	0.981	0.271	0.229	1.444	1.146
me	an 55	536 3	33 571	8 857	2 107	11.064	63 5	23.929
								4.705
2 64	5.0	155 4						0.889
	me sd		sd 5.635	sd 5.635 4.346	sd 5.635 4.346 3.71	sd 5.635 4.346 3.71 0.673	sd 5.635 4.346 3.71 0.673 1.257	

IgM Alb β Class Sample Statistic IgG IgA α_1 α_2 γ (mg%) size (mg%) (mg%) (%) (%) (%) (%) (%) (%) 1519.3 199.65 114.35 73.985 3.095 3.135 4.83 14.885 mean 1 20 sd 199.158 19.132 28.079 5.513 1.28 1.042 1.36 4.62 0.286 0.233 0.304 1.003 se 44.533 4.278 6.279 1.233 2870.333 182.6 129.067 58.74 3.68 4.353 8.267 24.867 mean 2 15 sd 277.906 19.231 24.845 6.706 1.574 2.513 3.374 6.182 71.755 4.965 6.415 1.732 0.407 0.649 0.871 1.596 se 2777.96 190 67.844 3.792 3.74 135.84 5.084 19.48 mean 410.31 1.406 1.484 1.606 3.417 3 25 sd 15.773 33.308 3.86 se 82.062 3.155 6.662 0.772 0.281 0.297 0.321 0.683 mean 2608.393 199.07 131.286 67.054 3.604 4.136 6.971 18.339 4 28 sd 267.147 16.873 22.247 5.046 1.125 1.527 2.125 3.693 50.486 3.189 4.204 $0.954 \quad 0.213 \ 0.289 \ 0.402 \ 0.698$ se

Summary Table: 1C

B: Analysis of Variance

In this section, we will study whether or not the means of 21 blood parameters, BP (Systol/Diastol) and pulse rates are different for 4 classes of people described above. For this study, we consider the following two way analysis of a variance model using class and sex as two classifying factors for each of the 21 blood parameters, pulse rate and BP (Systol/Diastol):

(1)
$$y_{i,j,k}(t) = m + a(i) + b(j) + c(i,j) + e_{i,j,k}(t)$$

where $y_{i,j,k}(t)$ = response obtained from the k_{th} individuals for the j_{th} (j = 0, 1) sex of the i_{th} (i = 1, 2, 3, 4) class, corresponding to the parameter, *t*.

m = general effect a(i) = effect due to i_{th} class (i = 1, ..., 4) a(j) = effect due to j_{th} sex (j = 0, 1)a(i, j) = interaction effect between i_{th} class and j_{th} sex

Assumptions of the Analysis of variance model:

(a)
$$\sum_{i} a(i) = \sum_{j} b(j) = \sum_{i} c(i,j) = \sum_{j} c(i,j) = o$$

(b) e(i, j, k) = independently and identically distributed as normal variate with mean zero and variance σ^2 .

The assumptions of the model imply that $y_{i,j,k(t)}$'s are independently normally distributed with mean m + a(i) + b(j) + c(i, j) and variance σ^2 . Since the number of observations of the 4 classes (20, 15, 28) are different, unbalanced analysis of varince techniques are used. This is clearly explained with an example by Kshirsagar (1983). Following the analysis of variance tables, the effects of β , γ , IgG, Alb, Tp, Tch and Ech were found to be significant. This implies that the mean of each of the parameters β , γ , IgG, Alb, Tp,Tch and Ech is different for 4 classes. The computations for these parameters are shown in the tables (2A -2G).

				/	
Source	SS	df	ms	F-value	P-value
Class	123.106	3	41.035	8.141	0
Sex	0.303	1	0.303	0.06	0.807
Interaction	1.754	3	0.585	0.116	0.951
Error	403.26	80	5.041		

Table 2A: ANOVA (β)

Table 2B: ANOVA (γ)

Source	SS	df	ms	F-value	P-value
Class	627.839	3	209.28	10.509	0
Sex	39.157	1	39.157	1.966	0.165
Interaction	35.099	3	11.7	0.588	0.625
Error	1593.121	80	19.914		

Table 2C: ANOVA (IgG)

Source	SS	df	ms	F value	P value
Class	14318260	3	4772753.3	47.721	0
Sex	68669.954	1	68669.954	0.687	0.41
Interaction	65112.199	3	3360328.2	33.599	0.844
Error	8001077.2	80	100013.47		

Source	SS	df	ms	F value	P value
Class	1236.765	3	412.255	14.108	0
Sex	0.011	1	0.011	0	0.984
Interaction	31.364	3	10.455	0.358	0.784
Error	2337	80	29.222		

Table 2D: ANOVA (Alb)

Table 2E: ANOVA (TP)

Source	SS	df	ms	F-value	P-value
Class	2.212	3	0.737	3.892	0.012
Sex	0.033	1	0.033	0.172	0.679
Interaction	0.465	3	0.155	0.817	0.488
Error	15.159	80	0.189		

Table 2F: ANOVA (TCH)

Source	SS	df	ms	F-value	P-value
Class	13386.909	3	4462.303	13.466	0
Sex	1.986	1	1.986	0.006	0.938
Interaction	1477.278	3	492.426	1.486	0.225
Error	26509.82	80	331.373		

Table 2G: ANOVA (EC)

Source	SS	df	ms	F-value	P-value
Class	14398.88	3	4799.627	15.454	0
Sex	272.46	1	272.46	0.877	0.352
Interaction	249.905	3	83.302	0.268	0.848
Error	24846.723	80	310.584		

C. Correlation Matrix

The correlation matrix for 13 selected important parameters (viz. Alb, N,B,E, M, Hb, Tc, Tp, Tch, Ec), based on 88 observations obtained after combining 4 classes, are given in table 3. From table 3, we note that 'r' values of Ester Cholesterol (Ec) and Total Cholesterol (Tch), Bc and Alb are fairly high. But there is moderately high negative correlation between eosinophils (E) and neutrophils (N) and between neutrophils (N) and lymphocytes (L). Other correlations are quite low.

	Alb	Ν	L	Е	М	Hb	Тс	Bc	Тр	Tch	Fc	Ec
Alb	1											
Ν	0.0244	1										
L	0.2996	-0.6653	1									
Е	0.2996	-0.5307	-0.1506	1								
М	-0.452	0.06	0.1844	-0.2399	1							
Hb	0.2225	0.623	-0.1233	0.0466	-0.1339	1						
Tc	0.0945	0.571	-0.2196	0.0221	0.0337	0.1507	1					
Bc	0.0172	0.512	0.0443	-0.1387	0.0181	0.0709	0.0095	1				
Тр	0.1128	0.199	-0.005	0.2262	-0.1798	-0.0862	-0.053	-0.0983	1			
Tch	0.446	0.0666	-0.2467	0.2879	-0.4295	0.1672	0.087	-0.027	0.2972	1		
Fc	-0.125	0.0743	-0.0461	0.0439	-0.0533	-0.0817	-0.168	0.0101	0.0146	0.3247	1	l
Ec	0.6435	0.1071	-0.2366	0.2906	-0.4317	0.2567	0.0279	-0.0307	0.2676	0.7868	-0.226	51

D. Categorical Data Analysis

In this section we test whetheror not, there is any association between (i) blood group and class and (ii) sex and class. Both the computed chi-squares for class vs sex were found to be insignificant (details are given in tables 4A and 4B). Thus neither the blood group nor sex has been found to be significantly associated with population class.

Class	BG				
	0	а	b	ab	Total
Normal	4	7	1	8	20
Malarious	8	0	1	6	15
Febrile	8	7	3	7	25
Dormant	6	7	3	12	28
Total	26	21	8	33	88

Table 4A: Contingency table (Marginal subtable)

Computed $\chi^2 = 10.6316 < 16.919 = \chi^2$ (0.05, 9), BG = Blood Group

Class		Sex	
	males	females	Total
Normal	3	17	20
Malarious	4	11	15
Febrile	10	15	25
Dormant	7	21	28
Total	24	64	88

Table 4B: Contingency table (Marginal subtable)

Computed $\chi^2 = 6.361 < 7.815 = \chi^2 (0.05, 3)$

E. Predicting malaria infection

In this section, we try to find a method of classifying an individual in any of the 4 classes (Normal, Malarious, Febrile and Dormant) on the basis of the data collected on 23 blood parameters, Pulse rate, Blood pressure as well as sex and Blood group. From the categorical data analysis mentioned in section we note that Blood group and sex have no association with the class. In other words, sex and blood group cannot classify an individual in any of the four classes. But from the Analysis of variance technique given in section B, we note that Alb, Tp, Tch, Ech, β , γ , IgG are different for different classes. We already mentioned in section C that Tch and Ech are highly correlated. So we use Tch along with β , γ , IgG, Alb and Tp as classification variables. To classify an individual in any of the four classes, we compute L(x), discriminant function or Fisher's classification function for each of the four classes (Seber, 1984) for some given value of $x = (\beta, \gamma, IgG, Alb, Tp, Tch)$. So for each of the four classes, the discriminant function was computed by using the STATISTICA (discriminant analysis programme) assuming prior probabilities are the same for all the four classes (details are given in tables 5A and 5B). The discriminant functions are as follows:

- Class 1: $L_1(x) = -904.862 + 22.633\beta + 15.701\gamma + 0.006 \text{ IgG} + 16.115 \text{ Alb} + 44.378 \text{ Tp} 0.386 \text{ Tch}$
- Class 2: $L_2(x) = -928.602 + 23.594\beta + 16.262\gamma + 0.022 \text{ IgG} + 16.099 \text{ Alb} + 41.704 \text{ Tp} 0.450 \text{ Tch}$
- Class 3: $L_3(x) = -905.102 + 22.649\beta + 15.714\gamma + 0.021 \text{ IgG} + 15.867 \text{ Alb} + 41.172 \text{ Tp} 0.321 \text{ Tch}$
- Class 4: $L_4(x) = -922.172 + 23.180\beta + 15.820\gamma + 0.019 \text{ IgG} + 16.012 \text{ Alb} + 43.170 \text{ Tp} 0.364 \text{ Tch}$

Table 5A

STAT DISCRIM. ANALYSIS		Classification Functions	Grouping	CL (malaria status)
Variable	G1:1	G2:2	G3:3	G4:4
β	2.633	23.594	22.649	23.180
γ	5.701	16.262	15.714	15.820
IgG	0.006	0.002	0.021	0.019
Alb	16.115	16.099	15.867	16.012
Тр	44.378	41.704	41.172	43.170
Tch	-0.386	-0.450	-0.321	-0.364
Constant	-904.862	-928.602	-905.102	-922.172

Table 5

STAT. DISCRIM. ANALYSIS	Classification Matrix (malaria. sta) Rows observed classifications Columns: Predicted classifications					
	Percent	G1:1	G2:2	G3:3	G4:4	
Group	Correct	p=0.22727	p=0.17045	p=0.28409	p=0.31818	
G1:1	100.0000	20	0	0	0	
G2:2	86.6667	0	13	1	1	
G3:3	76.0000	0	0	19	6	
G4:4	75.0000	0	1	6	21	
Total	82.9545	20	14	26	28	

The method involves the assignment of assigning an individual with $x = (\beta, \gamma, \text{ IgG}, \text{Alb}, \text{Tp}, \text{Tch})$ to the class with the longest value of the discriminant function. For example, when $\beta = 1$, $\gamma = 8$, IgG = 2000, Alb = 60, Tp = 6.5 and Tch = 130, we have x = (1, 8, 2000, 60, 6.5, 130) and $L_1(x) = 460.556$, $L_2(x) = 447.604$, $L_3(x) = 463.269$, $L_4(x) = 459.573$. So, an individual having $\beta = 1$, $\gamma = 8$, IgG = 2000, Alb = 60, Tp = 6.5 and Tch = 130 will be classified in class 3 (Febrile) since $L_3(x) = 463.269$ and is the maximum. In the process of classification, there is the possibility of misclassification. The estimated percentages of correct classification were computed by STATISTICA and it yielded 82.9545\% for the total and 100\%, 86.6\%, 76\% and 75\% respectively for class 1, class 2, class 3 and class 4 respectively.

Remark 1: Seshadri *et al.* (1983), Sharma *et al.* (1983) indicated the significant role of Albumin and Total Cholesterol in detecting malaria infected cases.

F. Multiple regression

Dependent	Independent	Multiple regression
Variable	Variable	
WBC	N,L,E,M	WBC=10594.4-35.69N-44.03L
		+46.33E-120.83M
RBC	Hb, Tc, Bc, IgG,	RBC=0.13+0.365 Hb* -0.0006 Tc
	IgM, IgA	+0.002 Bc +0.00002 IgG
		-0.00009 IgM -0.0009 IgA
Тр	Alb, α_1 , α_2 , β , γ	Tp =2.303+0.051 Alb*
		$+0.035\alpha_1+0.045\alpha_2 \ +0.046\beta \ =0.042\gamma *$
Tch	Fc,Ec	Tch=14.260+0.874Fc* +0.8823 Ec*

In this section, we have presented multiple regression for some of blood parameters discussed above.

* indicates significant (at 5% level) regression coefficient corresponding to the respective independent variable.

From the above table, we note that none of the independent variables N, L, B, M can be used effectively for predicting size of WBC since regression coefficients corresponding to the variables are independent. In a similiar fashion we we can conclude that only Hb, Alb, γ and both Ec and Bc can be used as reliable predictor for RBC, Tp, and Tch respectively.

4. CONCLUSION

The survey based on cross-sectional data analysis on the tribal population described in this paper revealed that the 5 blood parameters viz. Tp, Alb, Tch, IgG, β and γ , out of the 21 blood parameters considered, may be used for predicting malarial endemicity of the tribal community. The factors like blood group and sex do not take important roles for the prediction of malaria. There are fairly high positive correlations between Tch and Ec: Alb and Ec. But there are also moderately high negative correlations between R and N and M. The study is based on limited data. More accurate model making require the collection of further data, especially of females who are recalcitrant to respond due to their «social taboos».

ACKNOWLEDGEMENTS

We would like to thank the referee for his useful suggestions that greatly improved this work. We also thank Mr. A. Bose, Mr. P.P. Chakravorty and Mr. C. Kundu for statistical computations. Financial support was obtained from the Indian Council of Medical Research and the Indian Statistical Institute, Calcutta for conducting the survey.

REFERENCES

- [1] **Blood, B. Ed.** (1977). *In vitro methods in cell-mediated immunity*. Academic Press, New York.
- [2] Davie, J.V. and Lewis, S.M. (1984). Practical Hæmatology. Churchill Livingstone.
- [3] Kshirsagar, A.M. (1983). A course in Linear Models. Marcell Dekkar Inc., New York, 305–307.
- [4] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). «Protein measurement with Folin Phenol Reagent». *Journal of Biological Chemistry*, 193, 265–275.
- [5] Mancini, G., Carbonara, A.O. and Horemans, J.F. (1965). «Immunochemical quantitation of antigens by single radial immuno-diffusion». *Immunochemistry*, 2, 235.
- [6] Seber, G.A.F. (1984). Multivariate Observations. John Wiley & Sons, USA 332–335.
- [7] Sheshadri, C., Shetty B. Ramkrishna, Gouri, N., Sitaraman, R., Revathi, R., Venkatraghaban, S. and Chari, M.V. (1983). «Biochemical changes at different levels of parasitaemia in *Plasmodium vivax* Malaria». *Indian Journal of Medical Research*, 77, 437–442.
- [8] Sharma, S., Shrivastava, I.K., Amarnath Dutta, G.P. and Agarwal S.S. (1983). «Serum proteins and Immunoglobulin changes in Human Malaria». *Indian Journal of Malariology*, 20, 15–19.
- [9] Smithies, O. (1955). «Zone electrophoresis in starch gels: Group variations in the serum protein of normal human adults». *Biochemical Journal*, **61**, 629.
- [10] Wootton, I.D.P. (1974). *Micro-analysis in Medical Biochemistry*.4th ed. (J. and A. Churchill Ltd.) 83–86.